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Human Papillomavirus Virus-Like Particles Do Not Activate Langerhans Cells: A Possible Immune Escape Mechanism Used by Human Papillomaviruses

Steven C. Fausch, Diane M. Da Silva, Michael P. Rudolf, and W. Martin Kast

High-risk human papillomaviruses are linked to several malignancies including cervical cancer. Because human papillomavirus-infected women do not always mount protective antiviral immunity, we explored the interaction of human papillomavirus with Langerhans cells, which would be the first APCs the virus comes into contact with during infection. We determined that dendritic cells, normally targeted by vaccination procedures and Langerhans cells, normally targeted by the natural virus equally internalize human papillomavirus virus-like particles. However, in contrast to dendritic cells, Langerhans cells are not activated by human papillomavirus virus-like particles, illustrated by the lack of: up-regulating activation markers, secreting IL-12, stimulating T cells in an MLR, inducing human papillomavirus-specific immunity, and migrating from epidermal tissue. Langerhans cells, like dendritic cells, can display all of these characteristics when stimulated by proinflammatory agents. These data may define an intriguing immune escape mechanism used by human papillomavirus and form the basis for designing optimal vaccination strategies. The Journal of Immunology, 2002, 169: 3242–3249.

High-risk human papillomaviruses (HPV) are viruses that infect the epithelial layers of the oral, rectal, vaginal, and cervical mucosa. There is a causal link between cervical cancer and high-risk HPV infection (1–3). However, women with cervical cancer do not always initiate an immune response against HPV even though their lesions are positive for HPV (4–6). The development of an HPV vaccine is hindered by the fact that high-risk HPV particles cannot be produced in vitro. One vaccine strategy currently being used in clinical trials employs virus-like particles (VLP) (7, 8). VLP, consisting of the capsid proteins L1 or L1 and L2, are potent stimulators of immune responses against HPV capsid proteins without the need for adjuvant (9–12). Chimeric VLP, which have the E7 protein fused to either L1 or L2, are being developed for immunotherapeutic vaccine strategies against cervical cancer (3). The constitutive expression of high-risk HPV E6 and E7 proteins, which bind and inactivate the tumor suppressors p53 and pRb, respectively, in cervical cancer cells, makes them attractive targets for immunotherapy (11, 12).

The dendritic cell (DC) lineage comprises a family of cells that are the most professional APCs. DC subsets, with different morphology, phenotype, and function, are present at various sites within the body (13–15). Immature DC found in peripheral non-lymphoid organs are able to acquire and process Ag, thereby becoming activated, which results in a dramatic change in their cell surface and functional phenotypes (16). DC migration to regional lymph nodes results in the interaction with naive T cells, initiating an Ag-specific immune response (17–19).

Only a small number of activated DC are required for initiation of a potent immune response (15). Therefore, the activation status of the DC appears critical for the development of a primary immune response. In both in vitro and in vivo systems, immature DC can be activated by bacterial components such as LPS, inflammatory cytokines such as TNF-α and IL-1β, or receptor-mediated events such as the engagement of CD40 (20, 21). When activated, DC up-regulate MHC class I and II molecules, the costimulatory molecules CD80 and CD86, and the chemokine receptor molecule CCR7, and they increase the secretion of the cytokine IL-12 (IL-12 p70), all of which serve to facilitate the priming of naive CD4+ T cells and CD8+ cytotoxic T cells (16).

Langerhans cells (LC) are a unique subset of epidermal DC that have been extensively studied. LC form a three-dimensional network in the epidermis of skin and the epithelial layers of oral, rectal, vaginal, and cervical mucosa (15). TGF-β1, produced by epidermal keratinocytes, is thought to be essential for the development of LC in vitro from CD14+ cells (22, 23). In vitro, culturing human monocyte precursors with GM-CSF and IL-4 will differentiate them into cells displaying characteristics of DC. However, adding additional TGF-β1 to the cytokine milieu results in the generation of cells displaying characteristics of epidermal LC (22, 24). Epidermal LC differ from DC in that they express high levels of the adhesion molecule E-cadherin, which mediates their adhesion to keratinocytes in the epidermis, and the C-type lectin Langerin. Langerin is involved in the trafficking of extracellular multimannosylated ligands through Birbeck granules to MHC class II compartments (25). The presence of the rod or racket-shaped intracytoplasmic Birbeck granules also defines an LC, although the function of the granules is largely unknown (25–27). Epidermal LC also do not express the DC-specific ICAM-grabbing nonintegrin, or the receptors for heat-shock proteins 60 and 70 (28, 29). The presence, or lack thereof, of certain receptors on specific
subsets of DC indicates that the distinct subsets may react differently to various Ags.

We previously reported that in vitro derived human DC are capable of binding, internalizing, and becoming activated by HPV16-L1L2 VLP. DC incubated with chimeric HPV16-L1L2-E7 VLP are able to induce an E7 epitope-specific human T cell response in vitro (30). The knowledge that HPV infects through the epithelial layers and that LC are at the primary sites of infection prompted us to determine whether human monocyte-derived LC are capable of initiating an immune response against HPV VLP similar to DC. The results demonstrate that LC are able to bind and internalize HPV VLP in a manner similar to DC; however, in contrast to DC, they do not become activated. LC are unable to initiate an E7-specific T cell response in vitro after incubation with chimeric HPV16-L1L2-E7 VLP, whereas DC induce an HLA-restricted, E7-specific T cell response. Consistent with these in vitro findings, mouse LC were not induced to migrate out of the epidermal layer in response to HPV VLP in an ex vivo whole organ skin explant assay, although they could be activated and triggered to migrate by other stimuli. These data provide a possible explanation as to why HPV VLP are highly immunogenic as vaccines, whereas some women infected with wild-type HPV do not mount an effective immune response. HPV VLP when given as a vaccine are injected below the basal layers and therefore interact with DC, initiating an immune response. HPV itself infects through the suprabasal layers and interacts with LC, which may not initiate an immune response against the virus. Overall, these data suggest a possible means by which HPV may circumvent immune response induction.

Materials and Methods

Abs and reagents

Anti-HPV16 L1 mAb and Abs to human CD14 FITC; CD19 FITC; CD80 FITC; CD86 FITC; HLA-DR, DQ, DP FITC; CCR7; CD1a PE; streptavidin APC; and isotope controls were purchased from BD PharMingen (San Diego, CA). Goat anti-mouse FITC, goat anti-rabbit HRP, and goat anti-mouse IgG biotin were purchased from BioSource (Camarillo, CA). HLA-A, B, C PE was purchased from DAKO (Glostrup, Denmark). E-cadherin Ab was purchased from Zymed (San Francisco, CA). Abs for flow cytometric analysis were used at 1/50 dilutions in PBS containing 1% FCS and 0.01% NaN3. The human rIL-4 (rhIL-4) and rhGM-CSF were puriﬁed by ﬂow cytometric analysis were used at 1/50 dilutions in PBS containing 1% FCS and 0.01% NaN3. The human rIL-4 (rhIL-4) and rhGM-CSF were purchased from Peprotech (Rocky Hill, NJ) and stored according to manufacturer’s instructions. Western blot analysis was performed using 10 µg/ml CD40L for 1 h. Cells were subsequently incubated for 48 h in 15 ml complete medium containing 1000 U/ml rhGM-CSF. Cells were harvested, washed, and stained for flow cytometric analysis. Cells were stained for MHC class I, MHC class II, CD80, CD86, and CCR7. For binding experiments, cells were stained for flow cytometric analysis after a 1-h incubation with VLP at 4°C.

VLP uptake experiments

VLP were labeled with carboxyfluorescein diacetate (CFDA) (Vybrant CFDA Cell Tracer kit) for 4 h at room temperature. After this incubation, the preparation was washed with 4 l PBS/0.5 M NaCl overnight at 4°C. DC and LC were collected and incubated with 10 µg VLP-CFDA for 120 min at 37°C. At various time points, fractions were collected and fixed with paraformaldehyde. Flow cytometry measured the fluorescence of the DC and LC, resulting from uptake of labeled VLP. Uptake of VLP by DC and LC was detected by TEM.

IL-10 and IL-12 assay

For the IL-10 and IL-12 assays, 1.5 × 106 DC or LC loaded with VLP were incubated in 1.5 ml complete medium and 1.5 × 105 autologous T cells for 48 h. In control experiments, 1.5 × 106 DC or LC were loaded with 10 µg LPS for 48 h, then T cells were added and incubated for 5 min. The amount of IL-10 or IL-12 was determined using an IL-10 or IL-12 human (p70) ELISA protocol (Endogen, Woburn, MA). Statistical analysis was performed using Microsoft (Redmond, WA) EXCEL.

Mixed leukocyte reaction

For the MLR, 1.5 × 106 DC or LC loaded with VLP were incubated in 15 ml complete medium for 48 h. Cells were collected and washed twice with PBS, and 103 cells were put into each well of a 96-well U-bottom plate. A total of 105 autologous T cells was incubated with the DC or LC for 96 h at 37°C in a total volume of 200 µl. In control experiments, DC or LC were incubated without VLP or 10 µg LPS for 48 h, then T cells were added and incubated for 96 h. PHA (20 ng)-stimulated T cells served as a positive control for proliferation. After 96 h, 100 µl supernatant was taken and replaced with 100 µl of a 10 µCi [3H]thymidine/ml complete medium solution and incubated 12 h. Cells were harvested with a 96-well cell harvester, and incorporation of thymidine was measured using a TopCount Microplate Scintillation and Luminescence Counter (Packard Instrument, Meriden, CO), and statistical analysis was performed using Microsoft EXCEL.

In vitro immunization assay

In vitro immunization assays were performed using 1.2 × 106 DC or LC loaded with 10 µg chimeric HPV16-L1L2-E7 VLP for 1 h at room temperature, washed, and mixed with 25 × 105 autologous CD8+ T cells. Autologous CD8+ T cells were isolated from PBL by negative magnetic

DC and LC generation

Frozen PBL were thawed and washed once with RPMI 1640, containing 10 mM sodium pyruvate (Life Technologies, Gaithersburg, MD), 10 mM non-essential amino acids (Life Technologies), 100 µg/ml kanamycin (Sigma-Aldrich), and 10% FCS (HyClone, Logan, UT). This is referred to as complete medium. For DC, plastic adherent cells were selected by plating 200 × 105 cells in a 175-cm2 tissue culture flask for 2 h at 37°C. Nonadherent cells were washed away with PBS, and the remaining adherent cells were cultured for 6 days in medium containing 1000 U/ml rhGM-CSF and 1200 U/ml rIL4, of which one-half was replenished every other day. For LC, adherent cells were cultured for 6 days in medium containing 1000 U/ml rhGM-CSF, 1200 U/ml rIL4, and 10 ng/ml rTGFB-β1, of which one-half was replenished every other day.

Electron microscopy

For TEM, pellets of DC and LC were fixed with 4% glutaraldehyde prepared in 0.1 M cacodylate buffer and 1% osmium tetroxide, dehydrated using a graduated acetone series, and embedded in resin. Ultrathin sections were stained with uranyl acetate-lead citrate and analyzed with a Hitachi (Tokyo, Japan) H-600 (75 kV) electron microscope.

Activation and binding assay

For activation experiments, DC and LC were collected, washed twice with PBS, and incubated with VLP at a concentration of 10 µg/105 cells in 1 ml PBS for 1 h at room temperature. VLP are found to bind to the cell surface to a maximum within 1 h (32). For reactivation experiments, cells were incubated with VLP for 1 h and subsequently incubated with 1 µg/ml CD40L for 1 h. Cells were subsequently incubated for 48 h in 15 ml complete medium containing 1000 U/ml rhGM-CSF. Cells were harvested, washed, and stained for flow cytometric analysis. Cells were stained for MHC class I, MHC class II, CD80, CD86, and CCR7. For binding experiments, cells were stained for flow cytometric analysis after a 1-h incubation with VLP at 4°C.

Viral particle isolation

VLP were tested by transmission electron microscopy (TEM) for the presence of intact particles (31). Collecting the supernatants of sonicated insect cell pellets after centrifugation generated crude insect cell protein. VLP were tested by transmission electron microscopy (TEM) for the presence of intact particles (31). Collecting the supernatants of sonicated insect cell pellets after centrifugation generated crude insect cell lysates for control experiments. Limulus assay (Sigma-Aldrich) was used to detect and semiquantitate endotoxin in the preparations.

Donor material

PBL from healthy donors were obtained by leukapheresis. Leukocytes were purified by Ficoll gradient centrifugation (Nyncomed, Oslo, Norway) and stored in liquid nitrogen.

Mice

Female C57BL/6 mice, 6–8 wk old, were purchased from Taconic Farms (Germantown, NY) and kept under specific pathogen-free conditions.
depletion using hapten-conjugated CD4, CD11b, CD16, CD19, CD36, and CD56 Abs and a magnetic cell separator (MACS system) according to manufacturer’s instruction. Cells were cultured in 48-well plates (Costar, Cambridge, MA) at 0.5 × 10^6 cells/well in complete medium for 7 days at 37°C. Restimulations after 7 and 14 days were done with 0.5 × 10^6 cells/well of DC or LC, loaded with 10 μg/ml chimeric HPV16-L1L2-E7 VLP. DC and LC were subsequently washed with PBS, irradiated (25 Gy), and added to the cultures. For restimulations, the medium was supplemented with IL-2 at 50 U/ml at 2 and 4 days after restimulation. After 28 days, effector cells were pooled and tested for IFN-γ production by ELISPOT. Ninety-six-well multiscreen hemagglutinin plates (Millipore, Bedford, MA) were coated with 5 μg/ml anti-human IFN-γ Ab (BD PharMingen) overnight, washed, and blocked for 4 h with complete medium at 37°C. A total of 2.5 × 10^5 cells/well was incubated in the presence or absence of HPV16-E7 peptide aa 86–93 for 40 h at 37°C. Wells were washed and plates were incubated with biotinylated anti-human IFN-γ Ab (BD PharMingen) and avidin-alkaline phosphatase (Sigma-Aldrich). Spots were counted after staining with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. The average of the background counts was subtracted from the average of the sample counts. Statistical analysis was performed using Microsoft Excel.

### Ear explant assay

Ears were removed from naive mice, put into six-well plates with 3 ml complete IMDM, and incubated overnight at 37°C to remove endogenous cytokines. Epidermal sheets were prepared and dorsal ear halves were put into 24-well plates and incubated with 50 ng TNF-α, 5 μg LPS, or 5 μg HPV VLP. One mouse ear from each mouse was inoculated with TNF-α, LPS, or VLP. For an internal negative control for migration, the other ear from the mouse was inoculated with PBS. After 1 h at 37°C, wells were filled with 1 ml IMDM containing 10% FCS and incubated for 24 h at 37°C. Cells migrating into the culture medium were recovered by centrifugation, counted, and analyzed by flow cytometry for CD11c expression. Statistical analysis was performed using Microsoft EXCEL.

### Results

#### Generation of human monocyte-derived DC and LC

Because HPV infects through epithelial layers, we wanted to determine whether LC, which are resident in the epithelial layer, are capable of becoming activated and initiating epitope-specific CD8+ immune responses in a manner similar to DC when incubated with HPV VLP. Human monocyte precursors were incubated with GM-CSF and IL-4 to generate DC, and GM-CSF, IL-4, and TGF-β1 to generate LC. These results showed that the production of cells that have characteristics of DC and LC (Fig. 1). MHC class II+ large cells were gated and analyzed for the presence of specific cell surface markers (Fig. 1, A and B). Cells present were negative for CD3, CD14, CD19, and CD56, excluding the presence of T cells, B cells, NK cells, and monocytes/macrophages (data not shown). DC expressed low E-cadherin (Fig. 1C), while 70% of the LC expressed E-cadherin, a marker specific for human LC (Fig. 1D). Another hallmark of human LC is the presence of Birbeck granules (13). Birbeck granules were detected in the human monocyte-derived LC by TEM, but not in DC (Fig. 1E, data not shown).

### Binding and uptake of HPV VLP by human monocyte-derived DC and LC

The initial encounter of Ag and APC determines the outcome of the immune response. Therefore, we investigated the binding and uptake of HPV VLP by human DC and LC. Human DC and LC were generated, as described above, and tested for the ability to bind HPV VLP by flow cytometry. VLP bound to the surface of DC (Fig. 2A) and LC (Fig. 2B) equally well, suggesting that VLP can target both cell types.

Next we investigated whether DC and LC are equally competent at uptake of HPV VLP. VLP were labeled with the fluorescent marker CFDA. CFDA attaches to proteins and upon uptake it is cleaved by intracellular esterases, resulting in the generation of a fluorescent signal detectable by flow cytometry. DC or LC that have taken up labeled VLP will fluoresce. Labeled VLP bound to the surface of DC and LC similar to unlabeled VLP, indicating that labeling did not interfere with the initial binding interaction of VLP to APC (data not shown). CFDA-labeled VLP were taken up quickly by DC (Fig. 2C) and LC (Fig. 2D), with approximately the same kinetics. After 15 min, both DC and LC fluoresced significantly, with a maximum fluorescence achieved at ~120 min. DC and LC incubated with unlabeled VLP, and cells incubated with an excess of unlabeled VLP and labeled VLP did not fluoresce, indicating that the fluorescence was dependent upon the uptake of labeled VLP (data not shown). LC taken at the 15-min time point were fixed and assessed by TEM for uptake of VLP into intracellular vesicles. Endocytosed VLP were clearly visible within the LC at this time point, as shown in the inset picture (Fig. 2E). These data demonstrate that DC and LC are both able to bind to and take up HPV VLP.
Previously, HPV VLP were found to activate immature human DC to become mature DC (30). Now, we investigated whether HPV VLP can activate DC and LC equivalently, as assessed by the increased expression of cell surface activation markers. Activation of DC and LC by HPV VLP was compared with that of CD40L, a known activating agent commonly used in vitro for its ability to up-regulate various DC and LC surface markers. DC and LC were washed extensively to rid them of cytokines present in the growth medium, a critical step, as TGF-β has been previously found to inhibit the maturation of monocyte-derived human LC (22). Immature DC and LC were incubated with HPV VLP, CD40L, culture medium alone, or HPV VLP, followed by CD40L. After 48 h, cells were tested for up-regulation of cell surface activation markers by flow cytometry. Untreated DC already expressed reasonably high levels of MHC class I and II (Fig. 3A). After exposure to VLP, there was additional up-regulation of MHC class I and II, and up-regulation of CD80, CD86, and CCR7 on DC, similar to CD40L treatment. Untreated human LC also expressed reasonably high levels of MHC class I and II (Fig. 3B). After exposure to CD40L, LC up-regulated MHC class I and II, CD80, CD86, and CCR7. However, when LC were incubated with VLP, there was no additional up-regulation of MHC class I or II, nor up-regulation of CD80, CD86, or CCR7, indicating that HPV VLP were not able to activate human monocyte-derived LC. Incubation of DC and LC with LPS or TNF-α/IL-1β, other known activation stimuli, resulted in significant up-regulation surface markers (data not shown). However, incubation with disrupted VLP or purified L2E7 protein was unable to activate DC or LC (data not shown), confirming that the structure of the particle must remain intact for activation of DC (33). Subsequent incubation of HPV VLP-treated DC and LC with CD40L or LPS resulted in the up-regulation of surface expression markers (Fig. 3).

The activation of human monocyte-derived DC was not due to the presence of endotoxin in the VLP preparation nor due to another protein contaminant present as a result of the VLP production procedure. The endotoxin level in the VLP preparations was very low (0.085 EU/10 μg VLP, as determined by a Limulus assay). This level of endotoxin was not found to activate DC or LC (data not shown). Additionally, cell lysates of insect cells used to produce the VLP did not activate DC or LC (data not shown). These findings support the conclusion that HPV VLP are capable of up-regulating cell surface activation markers on DC, but not LC, and this inactivation can be reverted by treatment with other known activation stimuli.
HPV VLP selectively induce secretion of IL-12 p70 and proliferation of T cells by DC, but not by LC.

The secretion of IL-12 p70 is crucial for the development of Th1-type T cell responses (34). Therefore, because we found that human DC and not human LC up-regulated cell surface activation markers upon incubation with HPV VLP, we analyzed secretion of IL-12 p70 by both DC and LC after incubation with VLP. DC and LC were incubated with medium alone, medium plus LPS, or medium plus VLP. Supernatants were collected and tested for the presence of IL-12 p70 and IL-10 by cytokine-specific ELISA after 48 h. A low level of IL-12 p70 was secreted from untreated DC and LC (Fig. 4A). DC incubated with VLP or LPS secreted large amounts of IL-12 p70. In contrast to DC, LC did not secrete IL-12 p70 when incubated with VLP, but did secrete IL-12 p70 when incubated with the positive control LPS (Fig. 4A). TNF-α/IL-1β also induced DC and LC to secrete IL-12 p70 similar to LPS stimulation (data not shown). These data suggest that VLP activate human DC and promote a Th1-type T cell response, whereas this does not happen when VLP interact with LC. As IL-10 secretion by DC has been associated with an immunosuppressive phenotype (35), we measured IL-10 production in the supernatants obtained in this experiment. Neither DC nor LC increased secretion of IL-10 after incubation with HPV VLP (Fig. 4B), and therefore no immunosuppressive environment, at least as defined by IL-10 secretion, was induced by HPV VLP.

We then determined the capacity of the DC and LC to induce proliferation of T cells in an MLR by [3H]thymidine incorporation. DC and LC were incubated with VLP or LPS for 48 h, followed by incubation with T cells for 96 h. PHA-stimulated T cells served as a positive control for proliferation, which was set to 100%. T cells mixed with unstimulated DC or LC resulted in proliferation that was 31 and 38%, respectively, of the positive control (Fig. 4C). T cells incubated with DC or LC that had been incubated with LPS resulted in proliferation that was 95 and 83%, respectively, of the positive control. However, when DC or LC were incubated with VLP, DC induced proliferation that was 81% of the positive control, whereas LC induced proliferation that was only 41% of the positive control (Fig. 4C). As expected, unstimulated T cells did not show appreciable proliferation. These data indicate that DC incubated with VLP are capable of inducing a greater primary immune response of naive allogenic T cells than unstimulated DC, whereas LC incubated with VLP are not.
HPV VLP do not induce murine LC to migrate out of the epidermal layer of skin explants

In addition to up-regulation of activation markers, another hallmark of LC activation is migration out of peripheral tissues after encounter with Ag or proinflammatory cytokines. Therefore, we investigated whether murine LC are induced to migrate after interaction with HPV VLP similar to other known migration inducers such as TNF-α and LPS. Epidermal skin explants from naive mice were inoculated with PBS, TNF-α, LPS, or HPV VLP and incubated overnight at 37°C. The total number of migrating cells was counted, and the number of CD11c+ LC that had migrated out of the skin was enumerated by flow cytometry. CD11c expression was chosen to distinguish epidermal LC from cells of the monocytic lineage because CD11c expression is not regulated by VLP in vitro (data not shown). TNF-α and LPS inoculation of mouse epidermal skin increased the total number of cells migrating into the medium (Fig. 6A). In contrast, incubation of mouse epidermal skin with VLP did not increase the total number of cells recovered in the culture medium (Fig. 6A). Additionally, inoculation with HPV VLP did not induce an increase in the percentage of CD11c+ cells subsequently cocultured with autologous CD8+ T cells. Although LC are able to take up the VLP, they are unable to efficiently present to CD8+ T cells.

Induction of epitope-specific CD8+ T cell responses by DC loaded with chimeric VLP, but not LC

We next investigated whether the uptake of chimeric VLP by DC and LC would lead to the processing and presentation of peptides for the induction of epitope-specific, MHC class I-restricted T cell responses in vitro. Chimeric HPV16-L1L2-E7 VLP were used as immunogens, as they contain a well-characterized human HLA-A*0201-restricted epitope (E786–93) recognized by human CD8+ T cells (36). Human DC have been previously shown to initiate epitope-specific immune responses to this peptide when incubated with the chimeric HPV16-L1L2-E7 VLP (30). Therefore, these VLP were used to determine whether DC and LC are capable of endogenously processing Ag similarly.

DC and LC generated from PBL of healthy HLA-A*0201-positive donors were loaded with chimeric HPV16-L1L2-E7 VLP and subsequently cocultured with autologous CD8+ T cells. The cultures were restimulated twice with DC and LC, respectively, loaded with chimeric HPV16-L1L2-E7 VLP. Seven days after the last restimulation, the cells from each culture were collected and tested for a specific response to an HLA-A*0201-restricted E7-derived peptide by IFN-γ ELISPOT. HPV16-L1L2-E7 VLP-loaded DC were able to stimulate IFN-γ release from T cells in response to the E786–93 peptide similar to E786–93 peptide-loaded DC and LC cultures (Fig. 5). In contrast, there was no specific response against the E786–93 peptide when LC loaded with chimeric VLP were used for priming in vitro. This demonstrates that DC take up and process the VLP into peptides for presentation to CD8+ T cells. Although LC are able to take up the VLP, they are unable to efficiently present to CD8+ T cells.
dermis is induced by TNF-α (Fig. 6). These data indicate that LC migration out of the epidermis is induced by TNF-α, LPS, or HPV VLP. Cells migrating into the culture medium were counted and assessed for CD11c expression by flow cytometric analysis. The total number of migrating cells for each treatment is shown in A. The percentage of increase in CD11c+ cells compared with PBS-negative controls is shown in B. The data represent the mean ± SD of the results obtained (*, p < 0.01 by Student’s t test assuming homoscedastic, one-tailed distribution). The experiment was repeated three times with similar results.

FIGURE 6. HPV VLP do not induce murine LC to migrate out of the epidermal layer of skin explants. Ear skin explants were obtained from naive mice, and epidermal sheets were prepared. Epidermal sheets were inoculated ex vivo with PBS, TNF-α, LPS, or HPV VLP. Cells migrating into the culture medium were counted and assessed for CD11c expression by flow cytometric analysis. The total number of migrating cells for each treatment is shown in A. The percentage of increase in CD11c+ cells migrating compared with PBS-negative controls is shown in B. The data represent the mean ± SD of the results obtained (*, p < 0.01 by Student’s t test assuming homoscedastic, one-tailed distribution). The experiment was repeated three times with similar results.

Discussion

Our data show that both DC and LC are capable of binding and internalizing HPV VLP. Unlike DC, LC were not activated by these events, as seen by the lack of up-regulation of activation markers and the lack of increased IL-12 cytokine secretion. DC activated by VLP induced proliferation of autologous naïve T cell in an MLR, whereas LC incubated with VLP were unable to do so. The DC were also able to induce an HLA-A*0201-restricted E7-specific CD8+ T cell response after incubation with an E7-containing chimeric VLP, whereas LC did not. The inability of LC to activate T cells suggests that these cells remained immature, or unactivated, in the presence of a potent DC activator. Neither DC nor LC incubated with HPV VLP produced the immunosuppressive cytokine IL-10. This suggests that LC do not suppress the immune system when they encounter HPV VLP, but rather that they may not allow for an immune response to occur. Other factors could be contributing to the immunosuppressive phenotype, but these factors were not measured. It is unlikely that the LC were rendered functionally unresponsive to all stimuli after incubation with VLP, because the LC were able to respond appropriately to both LPS and TNF-α/IL-1β stimulation. Thus, it appears that the LC generated in vitro can respond to Ag, but do not receive an activation signal by HPV VLP.

Migration of LC from the epidermal layer usually occurs when the LC encounters and is activated by Ag. We tested HPV VLP-induced migration of epidermal LC in an ex vivo whole organ skin explant assay. This assay has previously shown that incubation of epidermal sheets with CpG motifs from bacterial DNA or TNF-α induces CD11c+ LC migration from the epidermis (37). Although there is no mouse counterpart to HPV, many laboratories test vaccine efficacy of HPV VLP and chimeric VLP using murine tumor models (3, 33, 38, 39). Therefore, mouse DC and LC may respond in a manner similar to human DC and LC in response to the virus capsid. Lenz et al. (40) have shown that HPV VLP are able to activate mouse DC; however, their study did not evaluate whether LC could respond similarly. We show that coculture of mouse epidermal ear skin and HPV VLP did not result in the migration of LC out of the ear epidermis. Although we could not evaluate DC migration in this system, it is clear that the LC, selectively, did not migrate in response to VLP, but did migrate in response to other classic stimuli, TNF-α and LPS. The defect responsible for the lack of response by LC may be in the signaling pathways initiated as a result of binding, differences in the routes of uptake or endocytosis, or different levels of receptor expression on LC vs DC. It is not clear whether the phenomena observed will stretch beyond HPV VLP. It will be interesting to see whether other viral particles act in a similar manner on DC and LC as HPV VLP.

Depending on the route of vaccination, in vivo immunizations with HPV VLP may result in very different outcomes. HPV VLP injected s.c. are potent stimulators of immune responses against HPV capsid proteins without the need for adjuvant (9–12). This may be a result of DC internalizing, processing, and presenting VLP Ags to naïve T cells. However, if HPV VLP are administered in the suprabasal layers, LC will be targeted. From our data, it is clear that LC will not be able to initiate a cellular immune response against VLP Ags. Therefore, HPV VLP vaccination may be best suited for injection s.c.

Other observations support the hypothesis that LC are unable to initiate a CD8+ T cell response to not only HPV VLP, but also intact HPV. The development of squamous intraepithelial lesions (SIL), the direct precursor to cervical cancer, is associated with a local Th type 2 cytokine profile, whereas there is an absence of a cell-mediated immune response, or Th1 response, which is preferred for tumor immunity (41–43). The development of SIL is associated with a relative increase in the LC chemoattractant macrophage-inflammatory protein 3α. TNF-α, in contrast, is decreased in SIL. These two factors may contribute to the increased density of LC in low-grade SIL. However, LC isolated from SIL have altered alloantigen presentation, resulting in the decreased ability to stimulate proliferation of T cells (44). These results, taken together, suggest that LC in SIL, the direct precursor to cervical cancer, are unable to initiate a cell-mediated response in response to intact HPV.

The targeting of DC and LC for inhibition of an immune response is not a quality unique to HPV. Percutaneous infection with Schistosoma mansoni leads to the activation of LC, but these cells remain in the epidermis. This results from the in situ production of PGD2 by either DC or LC. The PGD2 analog acting on the PGD2 receptor expressed by LC—a PGD2 receptor—impairs the emigration of LC from the epidermis and the development of contact hypersensitivity responses (45). Human melanoma cells inhibit LC differentiation from CD34+ precursors (46). Vaccinia virus, a poxvirus, is highly immunogenic, and vaccination strategies using this virus are used to eradicate smallpox (47). However, this virus abor- tively infects DC, blocks their maturation, and induces apoptosis to evade an immune response (48). Human CMV, adenovirus, and HIV encode proteins that affect Ag processing, formation of MHC class I and II molecules, and TAP functioning (49–51). It is therefore conceivable that HPV, which form persistent infections, have also evolved mechanisms to evade recognition by the immune system. The data presented in this study suggest that one such mechanism may be

HPV-LIKE PARTICLES DO NOT ACTIVATE LC

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